REMARKS

Claim Amendment

Claim 1 has been amended to specify that the promoter is a mammalian promoter and that it is capable of transcribing an siRNA molecule in mammalian cells. Support for this amendment can be found in paragraph 26 on page 7 of the application as filed.

Claims 3 and 17 have been amended to be consistent with the amendment to claim 1.

Claim 17 has further been amended to specify that the cell is a mammalian cell as originally set forth in claim 18.

Claim 18 has been canceled in view of the amendment to claim 17.

Claims 30-32 have been added. Claim 30 specifies a group of mammalian promoters by type. Claims 31 and 32 specify individual promoters within the types of promoters. Support for these new claims can be found in paragraph 26 on page 7 of the application as filed. These new claims read on the elected invention.

Applicants submit that none of these amendments constitute new matter, and their entry is requested.

Rejection under 35 U.S.C. § 103(a)

The Examiner rejected claims 1-9 and 17-23 under 35 U.S.C. §103(a) as being unpatentable over Engelke et al. (US 2003/0148519) in view of Livache et al. (US 5,795,715). Applicants traverse this rejection.

The Examiner cites Engelke et al. for its disclosure of a U6 promoter-containing siRNA expression cassette which further comprises a transcription termination sequence. This expression cassette is constructed by molecularly cloning. Engelke et al. further teaches a method of transfecting the cassette into mammalian cells. The Examiner directs attention to Figure 1A and the disclosure in Engelke et al. at paragraphs 17-20, 88-91, 124-126 and 140. The Examiner cites Livache et al. for its disclosure of a method of producing a double-stranded RNA expression cassette containing a promoter via a PCR-based method using oligonucleotide

primers that are complementary sequences that encompass the sequence of the promoter and the target sequence.

In view of the teachings of these two references, the Examiner contends that it would have been obvious to the skilled artisan at the time of the invention to use the PCR-amplification method of Livache et al. to make the U6 promoter containing siRNA expression cassette of Engelke et al. Applicants submit that the Examiner is in error in this rejection.

Engelke et al. disclose an expression cassette that includes a U6 promoter and an siRNA sequence. In addition to the passages cited by the Examiner, Applicants note that paragraph 195 states that the compositions including siRNAs, genes encoding at least one siRNA, expression cassettes encoding at least one siRNA, etc. are produced by any method well known in the art, such as chemical synthesis, PCR techniques or molecular cloning. Paragraph 196 of Engelke et al. then discusses the use of PCR to express functional U6 cassettes citing **Castanotto and Rossi (2002) in preparation** (emphasis added) and the cloning of such a cassette in a plasmid. Since this description cites the Castanotto and Rossi 2002 paper "in preparation," Applicants note that it is not likely that the Engelke et al. provisional application provides support for this disclosure and thus the effective date for this disclosure would be 14 November 2002, after Applicants effective filing date of 1 August 2002. Applicants also note that the "Castanotto and Rossi (2002) in preparation" is Applicants' manuscript describing the present invention. The only disclosures of the use of PCR in Engelke et al. are (i) to isolate the 7SL promoter from a vector for cloning into a new vector which contains a cloning site into which the siRNA gene could be inserted, (ii) to confirm the expression of the siRNA gene or (iii) to confirm the loss of RNA expression because of the expression of the siRNA from the expression cassette. Thus, there is no teaching in Engelke et al. of using PCR that is entitled to its provisional filing date to amplify a promoter sequence in which one of the primers for the promoter sequence further comprises an siRNA sequence.

Furthermore, Engelke et al., make their expression cassette by conventional cloning into an expression vector. The use of a cloning site results in a few extra nucleotides at the beginning of the transcript. For siRNAs and shRNAs these extra nucleotides at the 5' end of the transcript

dramatically reduce their functionality. The present invention does not use cloning sites or any cloning procedures. This fact provides for making a precise start (called +1) for the resulting RNA transcription which is not possible with the method of Engelke et al. Interestingly, when Engelke et al. references the present invention (i.e., Castanotto and Rossi (2002) in preparation), it is in the context of cloning the expression cassette into a plasmid which confirms that the Engelke et al. invention is the making of expression cassettes by conventional cloning.

In addition, Engelke et al., require and use a conventional expression vector to introduce the shRNAs in mammalian cells. The present invention is based on the direct use of PCR products. Importantly, these PCR cassettes are not based on the amplification of pre-existing sequences but are synthesized "*de novo*" by engineering the primers to contain the siRNA or shRNA sequences and the appropriate terminator. **To Applicants' knowledge, prior to the present invention, it was not known that a mini gene, such as the one created by PCR amplification, as described in the present application could be directly transfected in cells, be stable and produce a functional gene.**

Livache et al. teaches the use of two primers to prepare a double stranded DNA of a target nucleic acid. Each primer comprises a promoter sequence and a sequence that is complementary to the target nucleic acid sequence. The promoter sequence is the promoter of bacteriophage promoter, such as T7, T3 or SP6. PCR amplification then produces a double stranded DNA template which has a promoter sequence on the 5' end of each strand. The double stranded DNA template so produced has a double stranded promoter at each end of the cassette. The T7, T3 or SP6 promoter is a promoter recognized by a RNA polymerase and provides for transcription of the template sequence. The DNA template is transcribed by RNA polymerase to produce double stranded RNA. See, column 6, lines 12-23. Thus, Livache et al. does not teach PCR amplification of a promoter sequence in which the primers are complementary to a promoter sequence and one of the primers contains the sense or antisense strand of an siRNA. Instead, Livache et al. teaches that the primers are complementary to the target and contain the promoters at the 5' end of the primer. The primers do not bind to the promoter but bind to the target sequence. This is completely different than the present invention. In the present

invention, the primers are complementary to the mammalian promoter and one primer contains an siRNA sequence.

Furthermore, as previously discussed, Livache et al., amplify pre-existing target sequences by including a 22 nucleotides bacterial promoter (i.e., the T7, T3 or SP6 promoter) at the 5' end of the primers. This amplification results in a fragment that can exclusively be used for *in vitro* experiments and would not be functional in mammalian cells. Moreover, this design of Livache et al. cannot be applied to mammalian promoters because they are more than a hundred nucleotides in length – much larger than the promoters used by Livache et al. It was not possible with the technology available at the time of Livache et al., Engelke et al. and the present invention (in fact, it is still not even possible today) that mammalian promoters such as the Pol III U6 promoter could be included within a primer sequence for amplification of a target sequence as described by Livache et al. That is, a primer that would contain the Pol III U6 promoter at its 5' end as taught by Livache et al. would not function to amplify the target sequence which was the goal of Livache et al. because the resulting primer would be too large to achieve amplification of the target sequence. Mechanisms and designs for prokaryotic and eukaryotic transcription units are completely different, have different requirements and involve different polymerase in the process. The two systems cannot be compared. To clearly delineate the present invention from the teachings of the prior art, the claims have been amended to specify that the promoter that is being amplified by the primers in accordance with the claimed subject matter is a mammalian promoter.

In view of this analysis, it is clear that there is no motivation in the cited references to combine the cited prior art in the manner proposed by the Examiner. In addition, Applicants submit that the claimed subject matter would not result from the combination of references if such a combination was made. Specifically, there is no teaching or suggestion in Livache et al. to perform an amplification reaction with two primers that are complementary to a mammalian promoter sequence in which one of the primers also includes an siRNA sequence. The only teaching or suggestion in Livache et al. is to perform an amplification reaction with primers that are complementary to a target sequence in which both primers contain a promoter. There would

be no motivation to use the U6 promoter of Engelke et al. in place of the promoters used in Livache et al., because the resulting promoter-primer complex would not be functional to amplify the target sequence as done in Livache et al., because the U6 promoter is too large to include in a primer sequence. Thus, Applicants submit that the combination of Engelke et al. and Livache et al. does not render the claimed subject matter obvious.

In view of the above amendments and remarks, it is submitted that the present invention is not rendered obvious by the combination of Engelke et al. and Livache et al. Withdrawal of this rejection is requested.

Conclusion

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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